CONTRIBUTION TO A STUDY OF MICROSCOPICAL FUNGAL FLORA OF MOROCCO. II – *ALTERNARIA ALTERNATA* : MICROSCLEROTIA AND CHLAMYDOSPORES

by N. AMRANI and L. NAJIM*

SUMMARY. – The fungus isolated from a dark brown spot disease, developed on post-harvested apples (var. Golden), was cultivated in vitro and identified to *Alternaria alternata* (Keissler).

In old and dessicated cultures, maintained on 2 % YE medium, resting stages such as microsclerotia and chlamydospores, were observed for the first time in this species.

RÉSUMÉ. – Le champignon isolé à partir d'une pourriture brun foncé trouvée sur des pommes (var. Golden), après leur récolte, a été cultivé in vitro et identifié à *Alternaria alternata* (Keissler).

Dans de vieilles cultures désséchées, sur 2% YE, on a observé, pour la première fois dans le cas de cette espèce, des formes de résistance telles que les microsclérotes et les chlamydospores.

KEY WORDS : *Alternaria alternata*, chlamydospores, microsclerotia.

INTRODUCTION

During investigations of microscopical microflora, and studies of incidencies of this latest on Rosaceous cultures, in Middle-Atlas at Oulmes, at 800 m of altitude (NAJIM & al., 1984), we found numerous, usual or not usual fungi in this area, with sometimes a particular behavior in field or in laboratory, such as *Alternaria alternata* Keissler.

Among species of *Alternaria* there are many which have been reported to produce sclerotia or chlamydospores naturally or in culture :

– *A. padwicki* forms sclerotia in culture (ELLIS, 1971).

– *A. raphani* produces chlamydospores in culture (TABER & VANTERPOOL, 1963). ATKINSON (1953) suggested that the survival of this fungus in

* Laboratoire de Mycologie, Faculté des Sciences, Av. Ibn Batota, B.P. 1014, Rabat, Maroc.

dry soil cultures for a long period (5 years) is related to the formation of these chlamydospores.

– *A. longissima* forms chlamydospores on natural as well as artificial substrats (ELLIS, 1971).

The chlamydospores of these species are produced by the rounding-up of a cell or cells of essentially vegetative hyphae.

– Concerning *A. porri* f. sp. *solani*, when normal conidia are placed in natural soil, chlamydospores form within single cells of these conidia (BASU, 1971).

– *A. brassicae* produces conidial chlamydospores too (TSUNEDA & SKOROPAD, 1976).

The purpose of this paper is to describe the formation under certain cultural conditions, of hyphal microsclerotia and intraconidial chlamydospores.

MATERIAL AND METHODS

Strain origin :

A wild strain (noted 1b) of *A. alternata* is used. It was got from infected apples collected from fields at Oulmes (Morocco).

This fungus was also found on apples 24h - 48h after their going out from cold stores, where they were deposed for a few months.

Cultures

Cultures are realized in sterilized Petri dishes on solid medium composed of : 2 % yeast extract, 2 % glucose solution, and 2 % agar solution.

These cultures are incubated at 25°C (± 1°C) for more than 3 months. Usually, they are maintained on 2 % potato dextrose agar or 2 % malt agar medium.

RESULTS

Several *Alternaria* species have been reported to cause rounded dark brown rots on Golden apples (Fig. 1), inciting then substantial reduction in crop yield and being above all, a serious challenge to the prolonged storage of the fruits at low temperatures.

The observation of a conidial suspension of the fungus, isolated from infected apples, shows pluricellular spores, borne in long chains in culture, the majority with three to five cross septa and within the limits of 21-36 x 9,5 µm. It concerns *A. alternata* Keissler (Fig. 2). This has been moreover confirmed by the CBS (Baarn).

This strain of *A. alternata* cultivated on 2 % YE medium at 25°C (± 1°C) presents, in the centre of at least one-month-old cultures, among vegetative

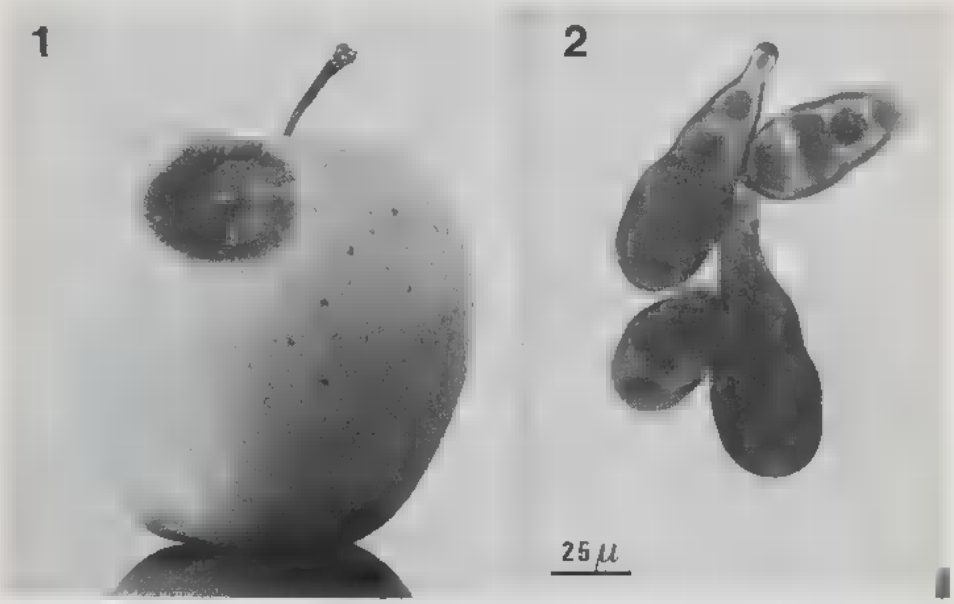

Fig. 1 : Apple (Var. Golden) presenting symptoms of alternariosis : a rounded dark brown and more or less large rot caused by *Alternaria alternata.*

Fig. 2 : Characteristical conidia of *A. alternaria* Keissler isolated from apple rot.

Fig. 1 : pomme (Var. Golden) présentant les symptômes d'une «alternariose» : pourriture arrondie de couleur brun foncé, causée par l'espèce *Alternaria alternata.*

Fig. 2 : Conidies caractéristiques d'*A. alternata* Keissler.

hyphae, pluricellular and at maturity, thick walled structures that are microsclerotia.

Young microsclerotia are formed initially by thin-walled cells that swell along hyphae (Fig. 3); then, several hyphae intermingle, the globose cells multiply and assembly (Fig. 4, 5), and their walls become thick and pigmented. Mature microsclerotia are firm, darkly pigmented, usually irregularly spheroidal and about 50 µm in diameter (Fig. 6).

When cultures are more than 3 months old they present, except for that hyphal microsclerotia, intraconidial chlamydospores (Fig. 7, 8). In these old cultures more or less desiccated, cytoplasm withdraws from some conidial cells and accumulates in one cell (Fig. 7), or two cells per conidium (Fig. 8), which develop thick cell walls and function as resting spores, or chlamydospores, that have about 4,5 µm in diameter. They are spherical and smaller than the cells within which they develop and their cell walls are transparent.

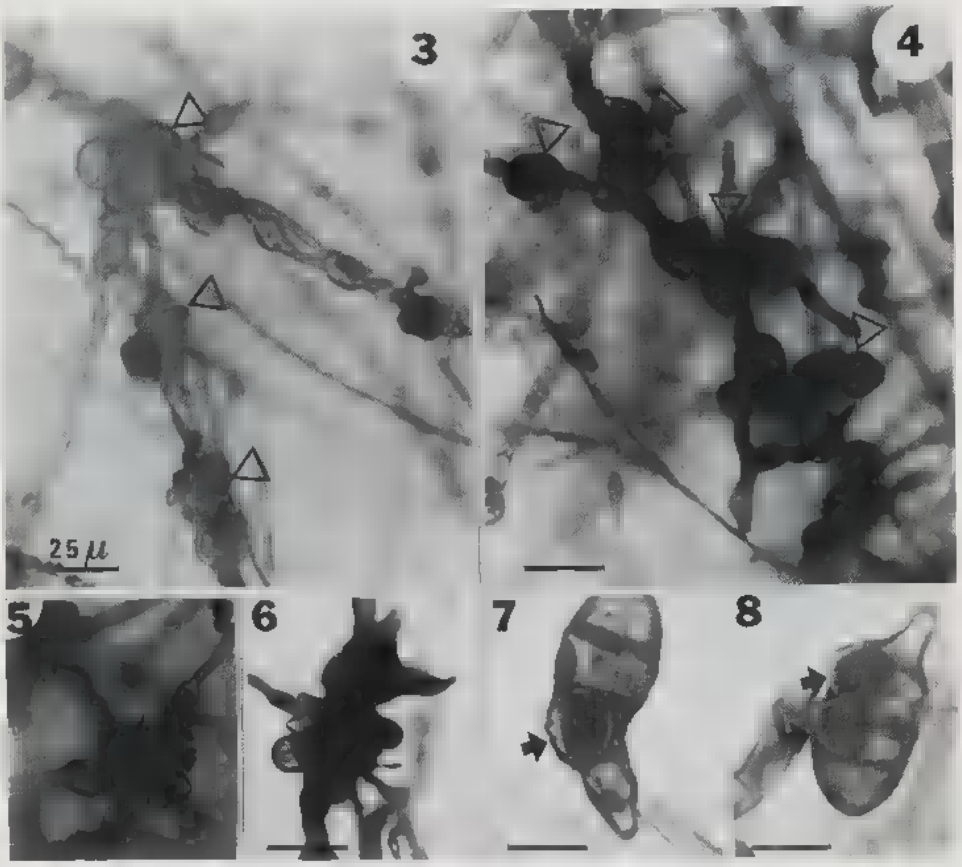

Fig. 3 : Initial stages in the formation of microsclerotia : Swollen hyphal cells more or less thin-walled and aggregated (arrows).

Fig. 4 : Globose cells of several intermingled hyphae aggregate and develop thick and pigmented cell walls (arrows).

Fig. 5 : Young microsclerotium consisting of less than 50 cells still identifiable.

Fig. 6 : Mature microsclerotium : firm and darkly pigmented.

Fig. 7 : *A. alternata* conidium presenting one endocellular rounded and thick-walled chlamydospore (arrow).

Fig. ■ : Two chlamydospores (arrows) inside ■ conidium.

Fig. 3 : Premières étapes dans la formation des microsclérotes : Cellules hyphales gonflées et plus ou moins aggrégées, à paroi relativement peu épaisse (flèches).

Fig. 4 : Des cellules gonflées de plusieurs hyphes entremêlées se rassemblent et développent des parois épaisses et pigmentées (flèches);

Fig. 5 : Microsclérote jeune constitué de moins de 50 cellules encore identifiables.

Fig. 6 : Microsclérote mûr : compact et pigmenté.

Fig. 7 : Conidie d'*A. alternata* présentant une chlamydospore arrondie et à paroi épaisse (flèche).

Fig. ■ : Deux chlamydospores intraconidiennes (flèches).

DISCUSSION AND CONCLUSIONS

The fungus isolated from the black rots on apples is identified as *A. alternata*. Its incidence as a postharvested pathogen of stored fruits has increased in recent years as a result of efforts to take advantage of marketing opportunities by prolonging the storage of fruits in Morocco.

Near the centre of a fungal colony (developed from a germinating spore) hyphae may often anastomose by thin branches which grow towards each other, this being a preliminary to the development of microsclerotia.

Most sclerotia are firm, frequently rounded masses of hyphae with or without the addition of host tissue or soil, and normally having no spores in them (AINSWORTH, 1971).

Some of the smallest structures thus formed, consisting of a few cells and without cortex and medulla, are described as microsclerotia, these structures often arise as masses of swollen and aggregated cells among vegetative intermingled hyphae.

These cells multiply either by producing septa, or by budding like *Verticillium alboatrum* (BROWN & WYLLIE, 1970) or *Pleiochaeta setosa* (HARVEY, 1975).

ANDERSON (1976) suggested that these morphological changes may result from a partial inhibition of the apical growth, which is responsible of the regular tubular form of the filaments.

Relating to *Alternaria brassicae* (TSUNEDA & SKOROPAD, 1976), microsclerotia are different, for they are conidia which aggregate in firm masses, the morphological and functional characteristics of which (rounded and darkly pigmented structures, resistant to desiccation and freezing) confer them the attribution of the term.

TSUNEDA & SKOROPAD (1976) suggest that their formation occurred under conditions unfavourable for vegetative growth, and as these structures withstood the effects of desiccation and freezing they might have a potential importance in the survival of the fungus in nature.

These writers got as well, naturally and in culture, endocellular chlamydospores in response to cold temperature (0-3°C) and gradual desiccation. This indicated that chlamydospores have too an importance in the survival of the fungus.

BASU (1971) got intraconidial chlamydospores in the same conditions, but this was for *Alternaria porri* f. sp. *solani*.

Other fungi have been reported to respond to nutrient depletion by forming chlamydospores (*Fusarium* sp.) or sclerotia (*Rhizoctonia*).

Concerning *Alternaria alternata*, microsclerotia arise essentially from vegetative hyphae, in one-month-old cultures, on YE, stored at about 25°C and subjected to gradual desiccation. Chlamydospores, in return, are formed in conidial cells, in cultures older than in case of microsclerotia and subjected to the same physical conditions.

Therefore, both structures are produced in old cultures on YE, in the centre of the colonies where nutritive substances are depleted. Their existence may be the result of a nutrient exhaustion, which is among a wide range of environmental and internal factors, like cellular ageing with production of autotoxic substances, freezing (TSUNEDA & SKOROPAD, 1976), of high temperatures (MARIAT, 1964). However, it isn't exactly known if this mode of development is hormonally controlled. CHET & HENIS (1975) thought that internal as well as external factors influence the initiation and the subsequent development of sclerotia.

The differentiation of structures like microsclerotia and chlamydospores is a new phenomenon for the species *Alternaria alternata* Keissler. It might be an ecological characteristic, related to the adaptation of this pathogen to the Moroccan climate, and particularly to Oulmes thermic condition, that are about + 40°C in summer and less than 0°C in winter.

It probably represents the means by which the fungus withstands the adverse conditions. These resting stages can persist for several years in soil, and present serious obstacles to the eradication of the parasite.

REFERENCES

AINSWORTH G.C., 1971 — Dictionary of the fungi 6th ed. Kew (England), Commonw. Mycol. Inst.

ANDERSON J.G., 1976 — Temperature-induced fungal development. *In* : J.E. SMITH & D.R. BERRY, *Filamentous Fungi*. London, Edward Arnold Publ. : 358-362.

ATKINSON R.G., 1953 — Survival and pathogenicity of *Alternaria raphani* after five years in dried soil cultures. *Canad. J. Bot.* 31 : 542-547.

BASU P.K., 1971 — Existence of chlamydospores of *Alternaria porri* f. sp. *solani* as overwintering propagules in soil. *Phytopathology* 61 : 1347-1350.

BROWN M.F. and WYLLIE T.D., 1970 — Ultrastructure of microsclerotia of *Verticillium albo-atrum*. *Phytopathology* 60 : 538-542.

CHET I. and HENIS Y., 1975 — Sclerotial morphogenesis in fungi. *Ann. Rev. Phytopathol.* 13 : 169-192.

ELLIS M.B., 1971 — Dematiaceous hyphomycetes. Kew (England), Commonw. Mycol. Inst.

HARVEY I.C., 1975 — Development and germination of chlamydospores in *Pleiochaeta setosa*. *Trans. Brit. Mycol. Soc.* 64 : 489-495.

MARIAT F., 1964 — Saprophytic and parasitic morphology of pathogenic fungi. *In* : H. SMITH & J. TAYLOR, *Microbial behaviour in vivo and in vitro*. London, Cambridge University Press : 85-111.

NAJIM L., CLAUZET J.P. and KADIRI M., 1984 — Contribution à l'étude de la flore fongique microscopique du Maroc - 1 - Le genre *Gonatobotrys*, quelques aspects physiologiques et morphologiques. *Cryptogamie, Mycol.* 5 : 109-120.

TABER R.A. and VANTERPOOL T.C., 1963 — *Alternaria* species on rape in western Canada. *Canad. Phytopathol. Soc. Proc.* 30 : 19.

TSUNEDA A. and SKOROPAD W.P., 1976 — Formation of microsclerotia and chlamydospores from conidia of *Alternaria brassicae*. *Canad. J. Bot.* 55 : 1276-1281.

ERRATUM :

Complément à l'article : «Contribution à l'étude de la flore fongique microscopique du Maroc - I - Le genre *Gonatobotrys* : quelques aspects morphologiques et physiologiques». *Cryptogamie, Mycol.* 1984 - 5 : 109-120.

«Les différentes espèces de *Gonatobotrys* sp. ont été déposées et répertoriées au Centraal bureau Voor Schimmelculture, sous les numéros suivants :

- *G. simplex*, CBS 466 84
- *G. africana*, CBS 465 84»